



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 31/59, C07C 403/00	A1	(11) International Publication Number: WO 93/14763 (43) International Publication Date: 5 August 1993 (05.08.93)
(21) International Application Number: PCT/US93/00796 (22) International Filing Date: 29 January 1993 (29.01.93) (30) Priority data: 07/827,173 29 January 1992 (29.01.92) US (71) Applicant: LUNAR CORPORATION [US/US]; 313 West Beltline Highway, Madison, WI 53713 (US). (72) Inventors: KNUTSON, Joyce, C. ; 24 North Prospect, Madison, WI 53705 (US). MORIARTY, Robert, M. ; 1030 Eric Street, Oak Park, IL 60302 (US). PENMAS- TA, Raju ; 493 West St. Charles, Elmhurst, IL 60126 (US). BISHOP, Charles, W. ; 3641 Okanogan Court, Verona, WI 53593 (US).		(74) Agents: GULBRANDSEN, Carl, E. et al.; 25 West Main Street, Suite 300, P.O. Box 2236, Madison, WI 53701-2236 (US). (81) Designated States: AU, CA, JP, NO, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: 1 α -HYDROXY-24-EPI-VITAMIN D ₄ (57) Abstract 1 α -Hydroxy-24- <i>epi</i> -vitamin D ₄ and novel intermediates formed in a novel method of preparing this compound. The method includes campesterol as a starting material which is converted to 24- <i>epi</i> -vitamin D ₄ which is in turn hydroxylated to 1 α -hydroxy-24- <i>epi</i> -vitamin D ₄ via tosylated and cyclic derivatives of 24- <i>epi</i> -vitamin D ₄ . 1 α -Hydroxy-24- <i>epi</i> -vitamin D ₄ has been found to be bioactive.		

BEST AVAILABLE COPY

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NL	Netherlands
BE	Belgium	GN	Guinea	NO	Norway
BF	Burkina Faso	GR	Greece	NZ	New Zealand
BG	Bulgaria	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SK	Slovak Republic
CI	Côte d'Ivoire	LJ	Liechtenstein	SN	Senegal
CM	Cameroon	LK	Sri Lanka	SU	Soviet Union
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	MC	Monaco	TC	Togo
DE	Germany	MG	Madagascar	UA	Ukraine
DK	Denmark	ML	Mali	US	United States of America
ES	Spain	MN	Mongolia	VN	Viet Nam
FI	Finland				

1 α -HYDROXY-24-EPI-VITAMIN D₄

5

TECHNICAL FIELD

This invention relates to biologically active vitamin D₄ compounds. More specifically, this invention relates to novel 1 α -hydroxy-24-epi-vitamin D₄ and a
10 method for preparing this compound as well as novel intermediates formed in the synthesis.

BACKGROUND

The vitamins D are a group of compounds that are steroid derivatives and are known to be important in the
15 regulation of calcium metabolism in animals and man. See, Harrison's Principles of Internal Medicine: Part Eleven, "Disorders of Bone and Mineral Metabolism, Chapter 335," E. Braunwald et al., (eds.), McGraw-Hill, New York, 1987, pp. 1860-1865.

20 The naturally occurring form of vitamin D in animals and man is vitamin D₃. Vitamin D₃ is synthesized endogenously in the skin of animals and man. In animals, including man, vitamin D₃ is activated by being hydroxylated in the C₂₅ position in the liver, followed
25 by 1 α -hydroxylation in the kidney to produce the hormone 1 α ,25-dihydroxy vitamin D₃. See, U.S. Patent No. 3,880,894.

1 α ,25-Dihydroxy vitamin D₃ is the hormonally active form of vitamin D₃. This hormone is taken up in the
30 intestine by specific cytoplasmic receptor proteins to stimulate calcium and phosphate transport from the intestinal lumen to circulation. The vitamin D₃ hormone also is taken up by specific cytoplasmic receptors in the parathyroid glands, the kidney, the osteoblasts, and
35 other target tissues, to elicit cellular responses

-2-

which, synergistically, stabilize blood levels of calcium and phosphorus, control the formation and removal of bone, and regulate the further production of 1 α ,25-dihydroxy vitamin D₃ itself. It is now recognized that the 1 α -hydroxy group is important in the binding of 1 α ,25-dihydroxy vitamin D₃ with its specific cytoplasmic receptors. It has also recently been reported that the vitamin D₃ hormones may play a role in cell proliferation and differentiation.

Vitamin D₂ is the major, naturally occurring form of vitamin D found in plants. Vitamin D₂ differs structurally from vitamin D₃ in that vitamin D₂ has a methyl group at C₂₄ and has a double bond between C₂₂ and C₂₃.

Considerable interest has focused on discovery and synthesis of various hydroxylated and dihydroxylated derivatives of vitamins D₃ and D₂. Examples of hydroxylated and dihydroxylated metabolites of vitamins D₃ and D₂ which have been found to occur naturally and/or have been synthesized include 25-hydroxy vitamin D₂, 24, 25-dihydroxy vitamin D₃, 25, 26-dihydroxy vitamin D₃, 1 α -hydroxy vitamin D₂, 23, 25-dihydroxy vitamin D₃, all of which have been found to exhibit vitamin D-like biological activity in vivo.

Unfortunately, while many of these active vitamin D metabolites held great promise as therapeutic agents, this promise has never been fully realized because of the extreme toxicity of these agents. For example, toxicity limits the efficacy of vitamin D₃, its active forms and analogs, to prevent bone loss or restore lost bone. Many studies indicate that at dosages required for these agents to be effective in bone loss prevention or restoration, hypercalcemia and hypercalciuria are serious problems. It has been reported that 1 α -hydroxy vitamin D₃ at a daily dose of 2 μ g/day (which has been shown in some studies to be effective in preventing loss of bone) causes toxicity in approximately 67 percent of patients.

-3-

Vitamin D₄ is a little known form of vitamin D. Vitamin D₄ was first described in 1936. See, Grab, W., Z. Physiol. Chem., 243:63 (1936); McDonald, F. G., J. Biol. Chem., 114:IVX (1936). See also, Windaus, A. and Trautmann, G., Z. Physiol. Chem., 247:185-188 (1937). Vitamin D₄, also known as irradiated 22,23-dihydro-ergosterol or 22,23-dihydro vitamin D₂ or 22,23-dihydroergocalciferol, differs from vitamin D₃ in that it contains a C₂₄ methyl group. The above cited references disagree as to the level of biological activity of this D vitamin, suggesting that in the rat, vitamin D₄ is one-third or three-fourths as active as vitamin D₃, and in the chick, either one-tenth or one-fifth as active as vitamin D₃.

In 1968, DeLuca et al. (Arch. Biochem. Biophys., 124:122-128 (1968)) confirmed that vitamin D₄ was less active than vitamin D₃. DeLuca et al. reported that vitamin D₄ is two-thirds as active as vitamin D₃ or vitamin D₂ in the rat, and one-fifth as active as vitamin D₃ in the chick.

DeLuca et al. make reference to the fact that "[t]he synthesis of vitamin D₄ has apparently been little used since it was first described by Windaus and Trautmann," and comment, "[t]his is perhaps due to the fact that vitamin D₄ is only of academic interest."

To applicants' knowledge, vitamin D₄ has remained "only of academic interest" as applicants are unaware of any further study of vitamin D₄ since that reported by DeLuca et. al. In fact, The Merck Index states with respect to vitamin D₄, "[i]ts biological activity seems doubtful." Merck Index, S. Budavari (ed.), 11th ed., Merck & Co., Rahway, N.J., (1989) pp. 1579, #9930.

There has been even less interest in vitamin D₄ analogues. Recently, however, a vitamin D₄ analogue, 1 α -hydroxy vitamin D₄, has been synthesized and shown to possess unexpectedly high biopotency and low toxicity (co-pending U.S. Patent Application Serial

-4-

No. 07/586,854, filed September 21, 1990). It was surprising to applicants in that application that this vitamin D₄ analogue had activity commensurate with the vitamin D₃ and D₂ hormones. Applicants, in this invention, have synthesized a related isomer of 1 α -hydroxy vitamin D₄ with equally surprising biological activity.

SUMMARY OF THE INVENTION

The present invention provides a stereoisomer of vitamin D₄, 1 α -hydroxy-24-*epi*-vitamin D₄, tosylated and cyclic derivatives of this compound, and a method of preparing these compounds.

In one aspect, the invention provides the compounds of formula (I) as defined hereinbelow. 1 α -Hydroxy-24-*epi*-vitamin D₄, the compound of formula (I) wherein R₁ and R₂ are each hydroxy groups, has been found to be bioactive. Other compounds encompassed by formula (I) have been found to be novel intermediates in the synthesis of 1 α -hydroxy-24-*epi*-vitamin D₄.

In another aspect, the invention provides the compounds of formula (II) which have also been found to be novel intermediates in the synthesis of 1 α -hydroxy-24-*epi*-vitamin D₄.

In further aspect, the invention provides a synthetic route for making the 1 α -hydroxy-24-*epi*-vitamin D₄. The method includes campesterol as a starting material which is converted to 24-*epi*-vitamin D₄ which is in turn hydroxylated to 1 α -hydroxy-24-*epi*-vitamin D₄ via tosylated and cyclic derivatives of 24-*epi*-vitamin D₄. A novel intermediate which is a derivative of campesterol has also been found.

Other advantages and a fuller appreciation of the specific adaptations, compositional variations, and physical and chemical attributes of the present invention will be gained upon an examination of the following detailed description of the invention, taken in conjunction with the accompanying drawings.

-5-

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will hereinafter be described in conjunction with the appended drawings, wherein like designations refer to like elements throughout and in which:

Figure 1 illustrates preparative steps for the synthesis of 24-*epi*-vitamin D₄ starting with campesterol; and

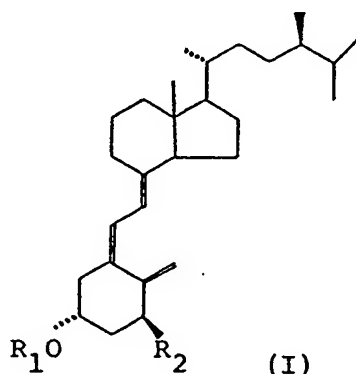
Figure 2 illustrates preparative steps for the synthesis of 1 α -hydroxy-24-*epi*-vitamin D₄ starting with 24-*epi*-vitamin D₄.

DETAILED DESCRIPTION

The present invention provides synthetic 1 α -hydroxy-24-*epi*-vitamin D₄ (1 α -OH-24-*epi*-D₄) as well as tosylated and cyclic derivatives of this compound.

As used herein, the terms "biological activity" or "biologically active" are meant to refer to biochemical properties of compounds such as affecting metabolism, e.g., affecting serum calcium concentration, or binding to an appropriate receptor protein, e.g., binding to vitamin D receptor protein. The term "*epi*" as used herein and as used generally in the art is meant to designate a different absolute configuration about a carbon atom, in the present invention, about the C₂₄ carbon, than in the parent vitamin D₄ structure.

In one of its aspects, the invention encompasses the compounds of the general formula (I):

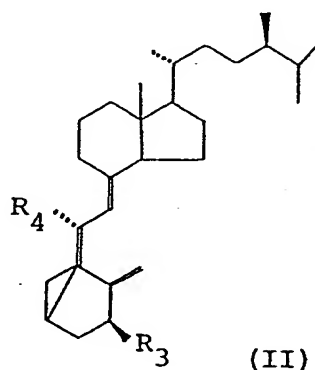


-6-

wherein R_1 is hydrogen or tosyl and R_2 is hydrogen or hydroxy, and salts, hydrates and solvates thereof.

Preferred among those compounds of formula (I) is that in which R_1 is hydrogen and R_2 is OH, i.e., 1 α -hydroxy-24-*epi*-vitamin D₄, which has been found to increase serum calcium.

In another aspect, the invention provides compounds of formula (II):



wherein R_3 is either hydrogen or hydroxy, and R_4 is methoxy, and salts, hydrates and solvates thereof. These compounds have been found to be useful and novel intermediates to form 1 α -hydroxy-24-*epi*-vitamin D₄.

In still another aspect, the invention involves the preparation of compounds of formulas (I) and (II) as well as another novel intermediate. Specifically, the synthesis of 1 α -hydroxy-24-*epi*-vitamin D₄, i.e., the compound of formula (I) wherein R_1 is hydrogen and R_2 is OH, is accomplished according to the schema presented in Figures 1 and 2. As seen in Figure 1, the synthesis uses the steroid campesterol as the starting material. Campesterol is available according to the procedure of Tarzia et al., *Gazz. Chem. Ital.*, vol. 97, pp. 102-106 (1967). Campesterol undergoes C₇ bromination, C₇-C₈ dehydrobromination in a four-step process to yield 7-dehydrocampesterol. The 7-dehydrocampesterol is then irradiated and thermally converted by methods well known

-7-

in the art to yield 24-*epi*-vitamin D₄ [also known as 22,23-dihydro-24-*epi*-ergocalciferol]. As seen in Figure 2, 24-*epi*-vitamin D₄ is then hydroxylated in a five-step process to yield 1 α -hydroxy-24-*epi*-vitamin D₄.

5 Specifically, campesterol is acetylated to form the 3 β -acetate. This campesterol acetate is subjected to C₇ bromination, C₇-C₈ dehydrobromination to form a double bond at C₇-C₈. The resulting 7-dehydrocampesterol acetate is then reduced to the novel
10 7-dehydrocampesterol. The 7-dehydrocampesterol is then irradiated and thermally converted to yield 24-*epi*-vitamin D₄. The 24-*epi*-vitamin D₄ is then tosylated to yield the 3 β -tosylate of 24-*epi*-vitamin D₄. The tosylate is displaced by solvolysis to yield the 6-methoxylate of
15 24-*epi*-3,5-cyclovitamin D₄. This 24-*epi*-cyclovitamin D₄ is subjected to allylic oxidation to form the 1 α -hydroxy 24-*epi*-cyclovitamin derivative. The 1 α -hydroxy 24-*epi*-cyclovitamin derivative is sequentially hydrolyzed and subjected to a Diels-Alder type reaction which removes
20 the 6-methoxy group and separates the 1 α -hydroxy 24-*epi*-vitamin D₄ (5,6 *cis*) from the 5,6 *trans* 1 α -hydroxy 24-*epi*-vitamin D₄. It is noted that the *trans* isomer, if desired, may be separated from the *cis* isomer via high pressure liquid chromatography according to the
25 procedure disclosed, for example, in U.S. Patent 4,719,204 issued to DeLuca et al.

1 α -Hydroxy-24-*epi*-vitamin D₄ has been found to possess physiological activity, namely, as an agent for increasing serum calcium concentrations. Specifically,
30 this compound increases serum calcium concentrations in rats with vitamin D deficiency. Thus, the compounds of the invention are potentially applicable to various clinical and veterinary fields, and are particularly useful for the treatment of abnormal metabolism of
35 calcium and phosphorus.

The following examples are to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. In the

-8-

following examples, all temperatures are set forth in degrees Celsius; unless otherwise indicated, all product yields are reported as percentages by weight. Proton nuclear magnetic resonance (^1H NMR) spectra were recorded with a Bruker AM-400 (400 MHz) with aspect 3000 Computer in CDCl_3 solutions with CHCl_3 as an internal standard. Chemical shifts are reported in ppm. Ultraviolet spectra were recorded with a Hitachi U-2000 Spectrophotometer and are reported for ethanol solutions.

Example 1: Synthesis of 1α -hydroxy-24-*epi*-vitamin D_3
Campesterol Acetate (2):

To a solution of 24.0 g (0.06 mol) of campesterol (1) in 180 ml of anhydrous pyridine was added 18.5 ml (0.196 mol) of acetic anhydride. The mixture was stirred at room temperature overnight and then 600 ml of water was added. The precipitate was filtered and washed three times with 200 ml portions of acetonitrile, and then air dried to yield 20.0 g (75%) of (2).

^1H NMR: (400 MHz, CDCl_3); δ ppm 0.7 (3H, s, 18- CH_3), 0.8 (6H, dd, 26 and 27- CH_3), 0.86 (3H, d, 21- CH_3), 0.92 (3H, d, 28- CH_3), 1.02 (3H, s, 19- CH_3), 2.04 (3H, s, OCOCH_3), 4.6 (1H, m, 3-H), 5.38 (1H, m, 6-H).

7-Dehydrocampesterol acetate (3)

A mixture of 10 g (0.023 mol) of (2), 4.56 g (0.016 mol) of dibromantin and 10.2 g (0.121 mol) of anhydrous sodium bicarbonate in 250 ml of dry hexane was heated under reflux in a nitrogen atmosphere for 2 hrs. The precipitate was filtered off and the solution was concentrated to dryness under reduced pressure. To the solution of the residue in 50 ml of anhydrous tetrahydrofuran was added 0.65 g (2.02 mmol) of tetrabutylammonium bromide, and the mixture was stirred at room temperature for 30 min under nitrogen. A solution of tetrabutylammonium fluoride (112 ml, 1M in

-9-

THF) was then added followed by 5.0 ml of s-collidine, and the mixture was stirred under nitrogen at room temperature overnight. To this reaction mixture was added ether (700 ml), and the organic phase was washed with water (2x200 ml), cold 1M HCl (2x200 ml) and 10% sodium bicarbonate (2x200 ml), and dried over anhydrous MgSO₄. Chromatography on silica gel with 10% ethyl acetate in hexane gave 5.5 g (55%) of (3).

¹H NMR: (400 MHz, CDCl₃); δppm 0.62 (3H, s, 18-CH₃), 0.80 (6H, dd, 26 and 27-CH₃), 0.86 (3H, d, 21-CH₃), 0.94 (3H, d, 28-CH₃), 0.96 (3H, s, 19-CH₃), 2.05 (3H, s, OCOCH₃), 4.7 (1H, m 3-H), 5.4 (1H, m, 7-H), 5.58 (1H, m, 6-H).

7-Dehydrocampesterol (4)

To a solution of 5.5 g (0.012 mol) of (3) in dry ether (500 ml) was added 3.38 g (0.089 mol) of lithium aluminum hydride. The mixture was stirred at room temperature for 2 hours, cooled with an ice water bath, and the reaction mixture decomposed by the cautious dropwise addition of ice water (5 ml). The mixture was filtered and the filtrate was concentrated in vacuo to remove most of the tetrahydrofuran. The residue was dissolved in 1000 ml of ether and washed with saturated NaCl solution (2x500 ml), dried over anhydrous MgSO₄ and concentrated in vacuo. The residue was purified on a silica gel column using 20% ethyl acetate in hexane to yield 4.0 g (80%) of (4).

¹H NMR: (400 MHz, CDCl₃); δppm 0.62 (3H, s, 18-CH₃), 0.8 (6H, dd, 26 and 27-CH₃), 0.86 (3H, d, 21-CH₃), 0.94 (3H, d, 28-CH₃), 0.96 (3H, s, 19-CH₃), 3.62 (1H, m, 3-H), 5.39 (1H, m, 7-H), 5.58 (1H, m, 6-H).

24-epi-Vitamin D₃ (5)

7-Dehydrocampesterol (4) (3.0 g, 7.5 mmol) was dissolved in 500 ml of ether and benzene (4:1) and irradiated with stirring under nitrogen in a water-cooled quartz immersion well using a Hanovia

-10-

medium-pressure UV lamp for 1.5 hrs. The solution was concentrated *in vacuo*, redissolved in 200 ml of ethanol and heated under reflux overnight. The solution was concentrated to dryness *in vacuo* and the residue was purified on a silica gel column using 20% ethyl acetate in hexane to yield 0.9 g (30%) of (5).

¹H NMR: (400 MHz, CDCl₃); δppm 0.54 (3H, s, 18-CH₃), 0.76 (6H, dd, 26 and 27-CH₃), 0.82 (3H, d, 21-CH₃), 0.9 (3H, d, 28-CH₃), 3.91 (1H, m, 3-H), 4.7 (1H, m, 19-H), 5.03 (1H, m, 19-H), 6.02 (1H, d, 7-H), 6.21 (1H, d, 6-H). UV (ethanol) λ_{max}: 265 nm.

24-epi Vitamin-D₃ tosylate (6)

To a solution of 0.9 g (2.26 mmol) of (5) dissolved in 10 ml of anhydrous pyridine was added 1.2 g (6.30 mmol) of tosyl chloride. The mixture was stirred under nitrogen at 5°C for 24 hrs. The reaction mixture was poured into 100 ml of cold saturated NaHCO₃ solution and extracted with ether (3x200 ml). The combined ether extracts were washed with 5% HCl solution (3x300 ml), saturated sodium bicarbonate solution (3x300 ml) and saturated NaCl solution (2x300 ml), dried over anhydrous MgSO₄ and concentrated *in vacuo* to yield 1.1 g (88%) of (6).

24-epi-3,5-Cyclovitamin D₃ (7)

To a solution of 1.0 g (1.81 mmol) of (6) dissolved in 100 ml of anhydrous methanol was added sodium bicarbonate 10.0 g (0.12 mol). The mixture was heated under reflux for 8 hrs. The reaction mixture was concentrated *in vacuo*. Water (200 ml) was added followed by extraction with ether (2x300 ml). The combined ether extracts were dried over anhydrous MgSO₄ and concentrated to dryness *in vacuo* to yield 600 mg (80%) of (7) as an oil.

¹H NMR: (400 MHz, CDCl₃); δppm 0.54 (3H, s, 18-CH₃), 0.78 (6H, dd, 26 and 27-CH₃), 0.86 (3H, d, 21-CH₃), 0.92 (3H, d, 28-CH₃), 3.25 (3H, s, -OCH₃), 4.16 (1H, d, 6-H),

-11-

4.86 (1H, m, 19-H), 4.98 (1H, d, 7-H), 5.02 (1H, m, 19-H).

1 α -Hydroxy-24-epi-3,5-cyclovitamin D₄ (8)

tert-Butyl hydroperoxide (1.13 ml, 3.39 mmol; 3M in
5 toluene) was added to a suspension of 95 mg (0.86 mmol)
of selenium dioxide in 65 ml of anhydrous
dichloromethane under nitrogen. The mixture was stirred
at room temperature under nitrogen for 3 hours. Then
0.13 ml of anhydrous pyridine was added followed by a
10 solution of 600 mg (1.45 mmol) of (7) dissolved in 20 ml
of anhydrous dichloromethane. The mixture was stirred
under nitrogen at room temperature for 15 min, then
25 ml of 10% NaOH solution was added and the mixture was
extracted with ether (3x100 ml). The combined ether
15 extracts were washed with 10% NaOH solution (3x100 ml),
water (3x100 ml), saturated sodium chloride solution
(2x100 ml), dried over anhydrous MgSO₄ and concentrated
to dryness in vacuo. The residue was purified on a
silica gel column using a mixture of 20% ethyl acetate
20 in hexane to yield 140 mg (23%) of (8) as an oil.

¹H NMR: (400 MHz, CDCl₃); δ ppm, 0.54 (3H, s,
18-CH₃), 0.79 (6H, dd, 26 and 27-CH₃), 0.88 (3H, d,
21-CH₃), 0.92 (3H, d, 28-CH₃), 3.24 (3H, s, -OCH₃) 4.2
(1H, m, 3-H), 4.21 (1H, d, 6-H), 4.94 (1H, d, 7-H), 5.15
25 (1H, m, 19-H), 5.21 (1H, m, 19-H).

5.6-cis and 5.6-trans-1 α -hydroxy-24-epi-vitamin D₄ (9,
10)

1 α -Hydroxy-24-epi-3,5 cyclovitamin D₄ (8) (110 mg,
0.26 mmol) was dissolved in 1.1 ml of dimethylsulfoxide
30 and 0.9 ml of acetic acid and heated at 50°C under
nitrogen for 1 hour. The solution was poured over ice
and 50 ml of saturated NaHCO₃ solution. The mixture was
extracted with ether (3x100 ml). The combined ether
extracts were washed with saturated NaHCO₃ solution
35 (3x100 ml), water (2x100 ml), and saturated NaCl
solution (2x200 ml), dried over anhydrous MgSO₄ and

-12-

concentrated *in vacuo* to yield the crude product 105 mg (95%) of (9) and (10).

5,6-cis-1 α -hydroxy-24-epi-vitamin D₄ (9)

To a solution of (9) and (10), 105 mg (0.25 mmol) in 5 ml of ethyl acetate, was added 20 mg (0.2 mmol) of maleic anhydride, and the mixture was stirred at 35°C for 24 hours under nitrogen. The solution was concentrated to dryness *in vacuo*. The residue was purified on a silica gel column using 40% ethyl acetate in hexane to yield 30 mg (28%) of (9).

¹H NMR: (400 MHz, CDCl₃); δ ppm 0.54 (3H, 1 s, 18-CH₃), 0.78 (6H, dd, 26 and 27-CH₃), 0.86 (3H, d, 21-CH₃), 0.92 (3H, d, 28-CH₃), 4.2 (1H, m, 3-H), 4.41 (1H, m, 1-H), 5.0 (1H, m, 19-H), 5.32 (1H, m, 19-H), 6.0 (1H, m, 7-H), 6.38 (1H, m, 6-H); UV (ethanol) λ_{\max} : 265 nm.

Example 2: Biological testing of 1 α -hydroxy-24-epi-vitamin D₄

Male weanling rats (Holtzman strain, Holtzman Company, Madison, Wisconsin) were fed a vitamin D deficient diet containing adequate calcium (0.47%) and phosphorus (0.3%). Within three to four weeks, this diet induces an extreme vitamin D deficiency characterized by low serum calcium and poor growth. After four weeks on this diet, the rats had serum calcium values less than 6 mg/dl. The rats were then separated into four groups and orally administered either 1 α -hydroxy-24-epi-vitamin D₄ in a vehicle such as coconut oil or the vehicle (control) for each of 14 days. Twenty-four hours after the last dose, the rats were killed, and the blood calcium measured by a standard laboratory technique. The results of these determinations are shown in Table 1.

-13-

Table 1

Increase in serum calcium concentration

	<u>Compound</u>	<u>Dose</u> <u>(mcg/kg/day)</u>	<u>Number</u> <u>of</u> <u>Rats</u>	<u>Serum Calcium</u> <u>Concentration</u> <u>(mg/100 ml)</u> <u>± Standard Deviation</u>
5	Vehicle	-	10	5.1 ± 0.42
	24- <i>epi</i> -1 α -OH-D ₄	0.042	11	5.8 ± 0.40
	24- <i>epi</i> -1 α -OH-D ₄	0.250	12	8.1 ± 1.25
	24- <i>epi</i> -1 α -OH-D ₄	1.500	12	10.5 ± 0.71

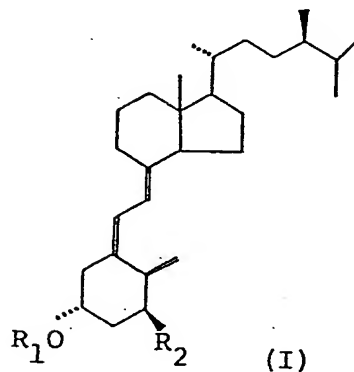
The data of Table 1 indicate that 1 α -hydroxy-24-*epi*-vitamin D₄ is effective at increasing serum calcium in the vitamin D deficient rat and that the response appears to be dose dependent.

While the present invention has now been described and exemplified with some specificity, those skilled in the art will appreciate the various modifications, including variations, additions, and omissions, that may be made in what has been described. Accordingly, it is intended that these modifications also be encompassed by the present invention and that the scope of the present invention be limited solely by the broadest interpretation that lawfully can be accorded the appended claims.

-14-

CLAIMS:

1. The compound of the formula (I):

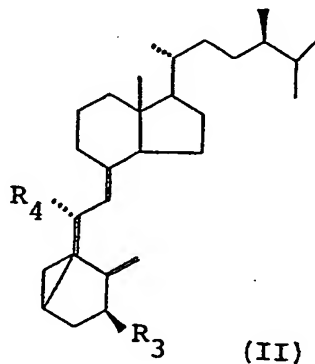


wherein R_1 is either hydrogen or tosyl and R_2 is either hydrogen or hydroxy, and salts, hydrates and solvates thereof.

2. The compound of claim 1, wherein the compound is 1 α -hydroxy-24-*epi*-vitamin D_4 .

3. The compound of claim 1, wherein the compound is 24-*epi*-vitamin D_4 tosylate.

4. The compound of the formula (II):



wherein R_3 is hydrogen or hydroxy and R_4 is methoxy.

-15-

5. The compound of claim 4, wherein the compound is 24-*epi*-3,5-cyclovitamin D₄.

6. The compound of claim 4, wherein the compound is 1 α -hydroxy-24-*epi*-3,5-cyclovitamin D₄.

5 7. 7-Dehydrocampesterol.

8. 5,6-trans-1 α -hydroxy-24-*epi*-vitamin D₄.

9. A method of preparing 1 α -hydroxy-24-*epi*-vitamin D₄, comprising:

- 10 (a) tosylating 24-*epi*-vitamin D₄ in the presence of dry pyridine to form 24-*epi*-vitamin D₄ tosylate;
- (b) solvolyzing 24-*epi*-vitamin D₄ tosylate to form 24-*epi*-3,5 cyclovitamin D₄;
- 15 (c) allylically oxidizing the 24-*epi*-3,5 cyclovitamin D₄ with selenium dioxide to form 1 α -hydroxy-24-*epi*-3,5-cyclovitamin D₄; and
- (d) hydrolyzing the 1 α -hydroxy-24-*epi*-3,5 cyclovitamin D₄ with a mixture of dimethylsulfoxide and an organic acid to form
- 20 an admixture of the 5,6 *cis* 1 α -hydroxy-24-*epi*-vitamin D₄ and 5,6 *trans* 1 α -hydroxy-24-*epi*-vitamin D₄ and forming a Diels-Alder adduct of the 5,6 *trans* 1 α -hydroxy-24-*epi*-vitamin D₄ to allow purification to yield 1 α -hydroxy-24-*epi*-
- 25 vitamin D₄.

10. A method of producing 24-*epi*-vitamin D₄ tosylate, comprising reacting 24-*epi*-vitamin D₄ with toluenesulfonyl chloride in the presence of dry pyridine.

30 11. A method of producing 24-*epi*-3,5-cyclovitamin D₄, comprising subjecting 24-*epi*-vitamin D₄ tosylate to buffered solvolysis.

-16-

12. A method of producing 1 α -hydroxy-24-*epi*-3,5-cyclovitamin D₄, comprising allylically oxidizing the 24-*epi*-3,5-cyclovitamin D₄ with selenium dioxide.

13. A method of producing 1 α -hydroxy-24-*epi*-vitamin D₄, comprising hydrolyzing the 1 α -hydroxy-24-*epi*-3,5 cyclovitamin D₄ with a mixture of dimethylsulfoxide and an organic acid to form an admixture of the 5,6 *cis* 1 α -hydroxy-24-*epi*-vitamin D₄ and 5,6 *trans* 1 α -hydroxy-24-*epi*-vitamin D₄ and subjecting the admixture to a Diels-Alder reaction forming an adduct of the 5,6 *trans* 1 α -hydroxy-24-*epi*-vitamin D₄ to allow purification to yield the 1 α -hydroxy-24-*epi*-vitamin D₄.

14. A method of producing 1 α -hydroxy-24-*epi*-vitamin D₄, comprising: oxidizing campesterol to form 7-dehydrocampesterol; irradiating the 7-dehydrocampesterol to form 24-*epi*-vitamin D₄; and hydroxylating 24-*epi*-vitamin D₄ to form 1 α -hydroxy-24-*epi*-vitamin D₄.

15. A method of producing 1 α -hydroxy-24-*epi*-vitamin D₄, comprising:

- (a) acetylating campesterol to form campesterol acetate;
- (b) oxidizing the campesterol acetate to form 7-dehydrocampesterol acetate;
- (c) reducing the 7-dehydrocampesterol acetate to 7-dehydrocampesterol;
- (d) irradiating and thermally converting 7-dehydrocampesterol to form 24-*epi*-vitamin D₄;
- (e) tosylating 24-*epi*-vitamin D₄ in the presence of dry pyridine to form 24-*epi*-vitamin D₄ tosylate;
- (f) solvolyzing 24-*epi*-vitamin D₄ tosylate to form 24-*epi*-3,5-cyclovitamin D₄;
- (g) allylically oxidizing the 24-*epi*-3,5-cyclovitamin D₄ with selenium dioxide to form 1 α -hydroxy-24-*epi*-3,5-cyclovitamin D₄; and

-17-

- 5 (h) hydrolyzing the 1 α -hydroxy-24-epi-
3,5 cyclovitamin D₄ with a mixture of
dimethylsulfoxide and an organic acid to form
an admixture of the 5,6 cis 1 α -hydroxy-24-epi-
6 vitamin D₄ and 5,6 trans 1 α -hydroxy-24-epi-
vitamin D₄ and forming a Diels-Alder adduct of
the 5,6 trans 1 α -hydroxy-24-epi-vitamin D₄ to
allow purification to yield 1 α -hydroxy-24-epi-
vitamin D₄.

FIGURE 1

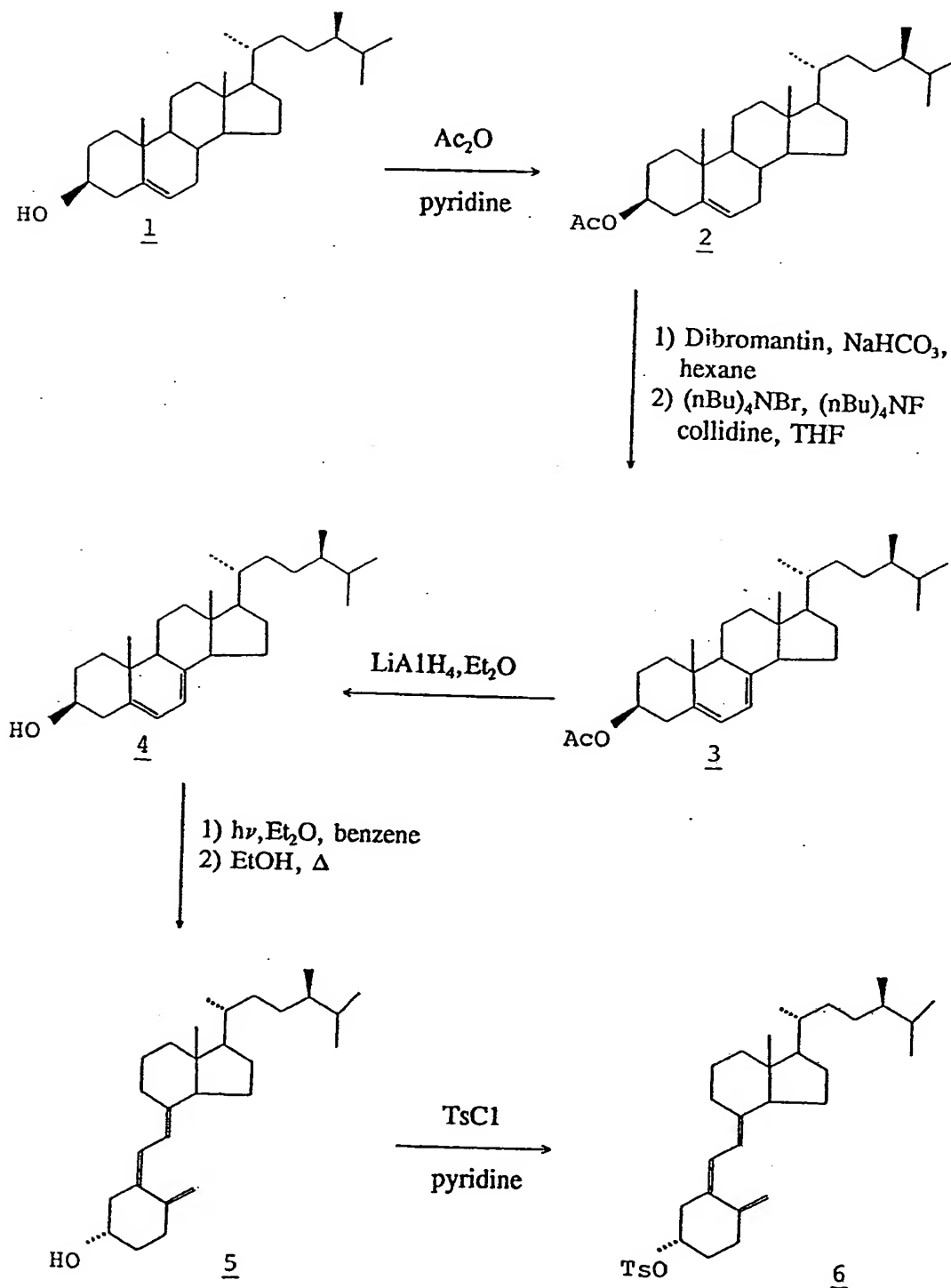
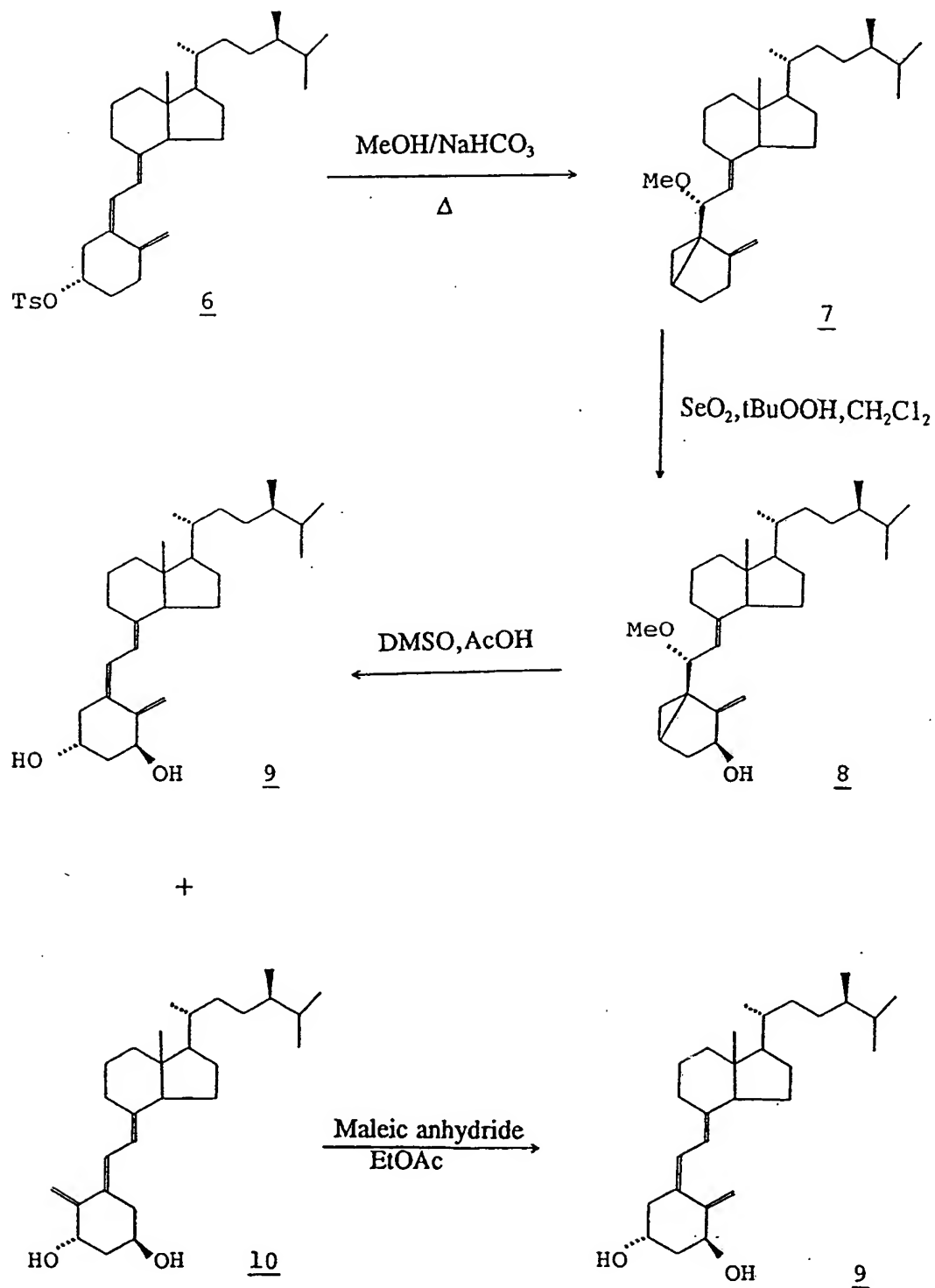


FIGURE 2



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/00796

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : A61K 31/59 C07C 403/00

US CL : 552/653; 514/167

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,769,181 (DeLUCA, ET AL.) 06 September 1988, See entire document.	1-6,8
Y	US, A, 4,973,584 (DeLUCA, ET AL.) 27 November 1990, See entire document.	1-6,8
Y	Archives of Biochemistry and Biophysics, 1968, (DeLUCA, ET AL.) "Synthesis, Biological Activity, and Metabolism of 22,23-H-Vitamin D4", pp. 122-128, esp. 122, 125-28.	1-8
Y	US, A, 4,448,721 (DeLUCA, ET AL.) 15 May 1984, See entire document.	8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	* T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
* A* document defining the general state of the art which is not considered to be part of particular relevance	* X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
* E* earlier document published on or after the international filing date	* Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
* L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	* &*	document member of the same patent family
* O* document referring to an oral disclosure, use, exhibition or other means		
* P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

04 MAY 1993

Date of mailing of the international search report

09 JUN 1993

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. NOT APPLICABLE

Authorized officer

KIMBERLY J. KESTLER

Telephone No. (703) 308-1235

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.

THIS PAGE BLANK (USPTO)